

Amendments to the Specification\:

Please replace paragraph [125] on page 48 with the following amended paragraph:

[0125] [0133] The amplification product was purified using the Qiagen gel purification kit, then digested with Sph I and Sac I overnight. The digested mixture was purified using Qiagen PCR purification kit, and the purified fragment was ligated into Sph I/Sac I-digested pRSET_B (Invitrogen) containing the cDNA sequence for ECFP and citrine (from Yellow cameleon 3.3). The construct was within the Bam HI/Eco RI sites of pRSET_B, and is behind a polyhistidine tag for bacterial expression. The resulting plasmid was amplified, sequenced and mutagenized using the QuickChange™ QUICKCHANGE™ site-directed mutagenesis kit (Stratagene) to introduce one amino acid change in the kemptide sequence, generating the plasmid C4kY2.1-pRSET_B.

Please replace paragraph [129] on page 48 with the following amended paragraph:

[0129] [0136] C4kY2.1- pRSET_B and C4kY2.1 (S475A)-pRSET_B each were transformed into E. coli strain BL21(DE3). A single colony was picked and grown in 100 to 500 ml LB medium containing 0.1 mg/ml ampicillin at 37 ° C. to an optical density of 0.4-0.8 at 600 nm, then induced with 0.1 mg/ml isopropyl thiogalactoside (IPTG) at 25 °C. for 12 to 24 hr. Cells were harvested by centrifugation, then the bacterial pellet was suspended in 4 to 10 ml B-PER™ II Bacterial Extraction Reagent (Pierce) and lysed by gentle shaking at 25 °C. for 15 min in the presence of protease inhibitors (Complete™ COMPLETE™ EDTA-free Protease Inhibitor tablet (Roche), 1 mM phenylmethylsulfonyl fluoride). The lysate was clarified by centrifugation at 12,000 g for 30 min at 4 ° C.

Please replace paragraph [130] on page 49 with the following amended paragraph:

[0130] [0137] Binding of the His₆ tag to Ni-NTA agarose (Qiagen) was carried out in a batch mode. The supernatant was filtered through a 0.22 µM syringe filter, then transferred to a new tube, to which 0.3 to 1 ml of the 50% (v/v) Ni-NTA slurry was

added. The suspension was mixed gently on a rotary shaker at 4 °C. for 1 hr. The lysate-Ni-NTA mixture was loaded into a column, which was washed with 10 volumes of TNS300 buffer (Tris-HCl, pH 7.4, 300 mM NaCl) and 10 volumes of TNS300 containing 10 mM imidazole. The chimeric protein was eluted with 1 to 3 ml of elution buffer (100 mM imidazole in TNS300) and dialyzed in TNS300 buffer at 4 °C. for 12 to 24 hr. When necessary, the protein was concentrated using a YM-30 Microcon MICROCON™ or Centricon CENTRICON™ concentrator (Fisher).

Please replace paragraph [134] on page 50 with the following amended paragraph:

[0134] [0141] For testing phosphatases, the PKA-phosphorylated chimeric reporter protein was concentrated using a YM-30 Microcon MICROCON™ concentrator and an equal volume of TNS300 buffer was added. The protein was then purified by Ni-NTA as described above. The purified protein was dephosphorylated with 2.5 U protein phosphatase 1 (PP 1; New England Biolabs) in the presence of 1 mM MnCl₂ in PP1 buffer (50 mM Tris-HCl, pH 7.0, 0.1 mM Na₂EDTA, 5 mM DTT, 0.01% Brij35) at 25 °C. for 30 min to 3 hr.

Please replace paragraph [139] on page 51 with the following amended paragraph:

[139] [0146] Cells were imaged on a Zeiss Axiovert microscope with a cooled CCD camera (Photometrics; Tucson Ariz.), controlled by Metafluor METAFLUOR 2.75™ software (Universal Imaging; West Chester Pa.). Dual-emission ratio imaging used a 440DF30 excitation filter, a 455DRLP dichroic mirror and two emission filters (480DF30 for ECFP, 535DF25 for citrine) altered by a filter changer (Lambda 10-2, Sutter Instruments, San Rafael, Calif.). Fluorescence images were background-corrected. Exposure time was 1000 ms and images were taken every 15 sec.

Please replace paragraph [168] on page 58 with the following amended paragraph:

[168]-[0174] For biochemical analysis of the reporter expressed in mammalian cells, mouse B82 cells, HeLa cells, or NIH3T3 cells in 10 cm dishes at 50-90% confluence were transfected with 1 μ g of the Eopt-pcDNA3 plasmid using **Effectene** **EFFECTENE™** (Qiagen) according to standard protocols. The cells were incubated with the DNA for 10 to 24 hr at 37° C. in 5% CO₂ and 10% calf (B82 cells and NIH3T3 cells) or fetal bovine serum (HeLa cells) in high glucose DMEM. The cells were then serum-starved in 0.5% calf serum (in high glucose DMEM) for 6 to 24 hr.